Immobilized PNGase as a tool for studying the biological role of glycans: influence of the support

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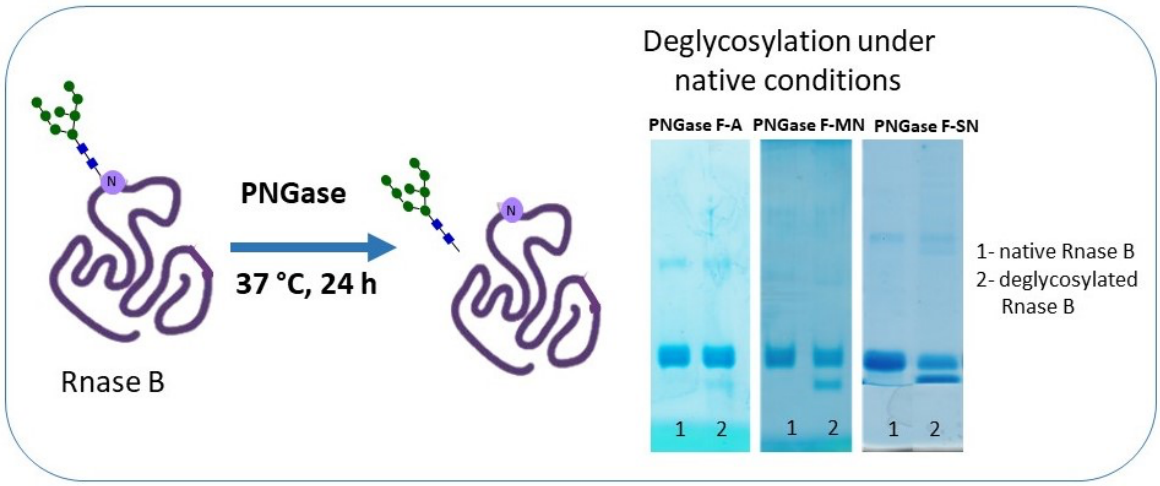
Carbohydrate-protein interaction between oligosaccharides from the cellular glycocalyx and protein receptors mediates several biological processes. Numerous technologies allow glycan structure elucidation, yet identification of their biological role is more complex. Enzymatic deglycosylation with immobilized PNGase is an interesting tool to confirm N-glycans role in a certain biological process. This enzyme catalyzes the hydrolysis of the bond between a glycoprotein asparagine and the internal GlcNAc. of the N-glycan. The fact that the enzyme is immobilized allow its removal upon deglycosylation so that changes in the biological function derived from the glycan removal can further be studied without its interference.

We immobilized PNGase F onto supports with different properties (agarose, magnetic and silica nanoparticles) using covalent strategies. In all cases immobilization yields above 80% were achieved with an expressed activity yield of 10%, allowing total removal of the N-glycan from RNase under denaturing conditions. Nevertheless N-glycan removal under native conditions, essential to perform further functional biological assays, was only possible with the enzyme immobilized onto nano-supports. Evaluation of N-deglycosylation of other model glycoproteins showed that magnetic nano-supports could not be used with iron containing proteins such as lactoferrin as they get stuck to the support. N-deglycosylation of epimastigote *Typanosoma Cruzi* was also evaluated. Successful results were obtained only when lysates were prepared in the presence of doxycholic acid.



Bibliographic references:
L. Bidondo, F. Festari, T. Freire, C. Giacomini (2022), Biotechnol. Appl. Biochem (69)209-220.